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LABORATORY FINDINGS IN THE LEUKEMIC STATES*

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Leukemia has been defined as a disease of the blood forming system, characterized by a tremendously elevated leukocyte count, and the textbooks often leave the erroneous impression that the leukocyte count generally exceeds 50,000 cells per c.mm. With newer staining methods and a closer study of cell types, coupled with a broader view of the manifiestations of the leukemic state, leukemia with only a moderate or even lowered leukocyte count is no longer considered a rarity. Leukemia, then, is more correctly defined as a condition characterized by a widespread growth of a single type of blood forming tissue and in some instances a reversion to embryonic blood formation. This growth may result in an increased number of leukocytes in the blood during certain stages of the disease, or the excess number of cells may be deposited in various body tissues and never appear in elevated numbers in the blood. The only clue to leukemia, from a laboratory point of view, may be in the presence of cell types of an abnormal and immature variety. Since these immature cells occur also in other conditions, they are considered only suspicious landmarks and are not always diagnostic of leukemia. The so-called "laboratory diagnosis" of leukemia, or any other blood condition, is viewed with suspicion by the expert and never attempted by the amateur.

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A review of the origin of the various types of leukocytes is an essential prelude to a classification of the various types of leukemia. There are three types of leukocytes recognized which in adult life have separate sites of origin and independent cycles of maturation; namely, the granulocytes (neutrophiles, basophiles, eosinophiles), lymphocytes (large and small), and monocytes (endotheliocytes, mononuclears, transitionals, etc.).

Granulocytes have their origin from the myeloid tissues of the bone marrow and are called the myeloid or myelocytic series of leukocytes. The precursor of this series is a large bone marrow stem cell, the myeloblast, which has a purple nucleus and a deeply-stained, blue cytoplasm, and is the only cell of this series which does not contain granules. As development proceeds, bright red granules make their appearance within the cytoplasm and when they are few in number the cell is called a premyelocyte. Following this stage the cytoplasmic granules increase and reach their highest peak of development. A majority of the premyelocytes remain neutrophilic in their reaction to polychrome dyes and are destined to become neutrophilic myelocytes as the granules increase in numbers. Others develop an acidophilic granule reaction and become eosinophilic myelocytes, while a few stain with the basic dyes and are called basophilic myelocytes.

Each type of myelocyte becomes the precursor of its respective mature form. The nucleus then becomes indented and assumes the juvenile form, then lengthens into a band, and finally segments. Myeloblasts, therefore, are the genetic progenitors of three types of mature granular cells; namely, the polysegmented neutrophil, the eosinophil, and the basophil. All leukocytes arise extravascularly, become motile, and enter the circulation through the capillary wall in the bone marrow. At the premyelocytic stage the granular cells develop an oxidizing ferment in their cytoplasm which results in a positive reaction to the peroxidase stain and the indophenol blue synthesis. These stains are used to aid in the identification of this series of cells.

Technic for the peroxidase stain, (Method of Sato and Sekiya).

Make a blood smear in the usual manner. Allow to dry in the air. Flood the slide for three minutes with Solution A (0.5 gm. copper sulphate in 100 c.c. distilled water). Pour off and without washing flood for two minutes with Solution B (0.2 gm. benzidin in 200 c.c. distilled water to which is added 4 drops of hydrogen peroxide; filter). Pour off excess and without washing, counterstain for two minutes with a 1 per cent aqueous solution of safranin. Wash thoroughly and dry in the air. The peroxidase positive granules of the granulocytes stain blue or blue-green and lymphocytes red. The nuclei of all cells are red. Red cells and platelets make a faintly pink, shadow-like background for the brilliantly stained granulocytes.

Indophenol Blue Synthesis. Modification of Schultze's Method.

Fix the blood film in a 40 per cent solution of formaldehyde plus absolute alcohol (equal parts). Allow to dry in the air. Flood slide for three minutes with Solution A. Wash with normal saline (gently). Flood slide for three minutes with Solution B and wash again. Counterstain for three minutes with a 1 per cent aqueous solution of sanfranin. Wash and dry in the air.

Solution A: 100 c.c. of a 1 per cent alphanaphthol in physiological solution of sodium chloride to which is added 1 c.c. of tenth normal sodium hydroxide.

Solution B: A 1 per cent solution of paradimethyl-paraphenylenediamine base (Merck) in a physiological solution of sodium chloride.

Myeloid and monocytic granules stain blue with this stain and those of the lymphocytes stain red.

Lymphocytes have their origin in the lymphoid tissue from a large, deeply staining, mononuclear cell, which is often indistinguishable morphologically from the myeloblast. This cell develops directly into the large lymphocyte which is found in normal blood. The bright red granules which appear occasionally in the large lymphocyte are different in structure and may be distinguished easily from the granules of the myeloid cells, both by appearance and by the peroxidase stain. As the large lymphocyte matures it decreases in size until the fully grown lymphocyte possesses only a small zone of cytoplasm and is then known as the small lymphocyte. Thus, it can be seen that the only difference between the large and small lymphocyte is one of age and there seems to be little necessity for separating them in the routine differential count. This entire series of cells reacts negatively to the peroxidase stain and the indophenol blue synthesis.

There is a divergence of opinion concerning the origin of the monocytes. Naegeli believes they are derived from myeloblasts, but most of American authors place this series in a separate cycle of maturation, tracing its origin from a stem cell, the monoblast, which in turn, arises from the reticuloendothelial system scattered throughout the body. This parent cell closely resembles the other leukoblasts.

The mature monocyte is the largest cell of the blood and frequently requires supravital stains for definite differentiation from other cell types, especially the juvenile form of the granular series. Both have a lobulated, indented or horseshoe-shaped nucleus with a granular cytoplasm. The zone of cytoplasm in the monocyte, however, is wider, more irregular and more delicately stained and the chromatin of the nucleus is lighter and less densely packed; the granules are smaller and have a more delicate pink-lavender color. Opinions are divided relative to the peroxidase reaction of mono-

cytes, but the majority seem to favor a peroxidase positive reaction for monocytic granules.

Osgood and Hunter offer evidence that the plasma cell is a normal constituent of the blood and bone marrow and that it is not derived from lymphoblasts, myeloblasts or megaloblasts, but is as distinct an entity as other leukocytes. They describe these cells as round or oval, with eccentrically placed nuclei, and ranging in size from 12 to 20 micra. The chromatin structure is coarse, with dense masses often arranged in a typical "cartwheel" fashion. The cytoplasm is abundant, deeply basophilic and non-granular with a well-defined, pale perinuclear zone. Some hematologists report the presence of plasma in differential counts in a large percentage of their patients. Others seem never to find them.

If there are three separate origins for leukocytes there is a possibility for the development of three types of leukemia; myeloid, arising in the bone marrow; lymphatic, originating in lymphoid tissue; and monocytic, resulting from a reticulo-endothelial overgrowth. Each of these conditions may assume a chronic or an acute form and may at any time undergo a sub-leukemic phase in which the leukocyte count is only moderately elevated, or an "aleukemic" phase in which the total number of circulating leukocytes is below normal.

We suggest the following classification of leukemia for purposes of laboratory work, which specifies the predominating cell type and the elevation or depression of the leukocyte count.

Laboratory Classification of Leukemias

1. Myelocytic leukemia (chronic).
   a. Leukemic phase (leukocyte count elevated).
   b. Aleukemic phase (leukocyte count below normal).
2. Myeloblastic leukemia (acute).
   a. Leukemic phase.
   b. Aleukemic phase.
3. Lymphoblastic leukemia (acute or chronic).
   a. Leukemic phase.
   b. Aleukemic phase.
4. Monocytic leukemia (acute or chronic).
   a. Leukemic phase.
   b. Aleukemic phase.
5. Plasma cell leukemia (acute or chronic).

The laboratory findings in the various leukemic states are fairly well defined, but emphasis should be placed, again, upon the danger of attempting a diagnosis based on laboratory findings alone. The blood picture and clinical findings combined often offer only presumptive evidence and the diagnosis remains obscure until the

disease has run its course and autopsy reveals the correct diagnosis. Clinical and autopsy findings may fail to clear up the doubt, unless accurate laboratory observations have been made, so that clinical and laboratory findings are necessary supplements to each other to form a unified criterion for diagnosis. ONE SHOULD NEVER "DIAGNOSE" A BLOOD DYSCRASIA IN THE LABORATORY.

Laboratory Findings in the Chronic Leukemias

The chronic type of leukemia, limited almost exclusively to individuals of middle and old age, is insidious in onset and the disease is frequently fully developed when the patient presents himself to the physician. For this reason, the blood picture is more clear cut and of more diagnostic aid to the clinician than that seen in the more acute forms.

In chronic myeloid leukemia, when the blood is examined after the disease is well advanced, there is usually a markedly elevated leukocyte count, 100,000 or more per c. mm., with 95 to 99+ per cent granulocytes. The differential count shows a predominance of myelocytes and mature segmented neutrophils with a smaller number of intermediate forms. All three types of myelocytes are present, but as a rule a single type predominates, usually the eosinophilic myelocyte. It is not unusual, however, for the myelocyte type to shift during the course of the disease. Premyelocytes and myeloblasts occur only in small numbers during the early and middle stages of the disease. All of these cells, with the single exception of the myeloblast, react strongly positive to the peroxidase and indophenol blue stains.

The condition is invariably accompanied by a progressively severe anemia, usually of the hypochromic and microcytic type, but as the bone marrow becomes over-crowded with leukocytes, erythropoietic tissue becomes damaged by compression. This may result in a macrocytic, hyperchromic, pernicious anemia-like red cell picture. Thus, if the blood is examined for the first time during this stage, and the process should be sub-leukemic, then one might easily mistake this for a pernicious anemia blood picture, illustrating again the danger of "laboratory diagnosis." Nucleated erythrocytes of all types are found at varying times with marked variation in the size, shape and staining properties of mature erythrocytes.

During the early stages of the disease the platelets may be increased in numbers due to the stimulation of the entire bone marrow by accelerated leukopoiesis. Later, however, thrombopoiesis becomes inhibited and platelets profoundly decreased, re-

sulting in the hemorrhages which accompany leukemia in its terminal stages.

As the disease progresses, the circulating leukocytes may become more immature with myeloblasts appearing in larger numbers until finally the patient may die in an acute phase with myeloblasts predominating. This occurs somewhat infrequently, however, since the patient is more likely to die from secondary infectious processes.

Chronic lymphatic leukemia differs from the myeloid form only in the type of cell involved. The blood typically shows an elevated leukocyte count, frequently, of several hundred thousand, with mature lymphocytes predominating. Lymphoblasts are not prominent in the picture unless the disease terminates in an acute phase. These cells react negatively to the peroxidase stain and the indophenol blue synthesis. Mature neutrophils are usually present in normal numbers, but are seen only occasionally on the stained smear. Smudges and degenerating lymphocytes are invariably found in great numbers, and in some cases, in a two to one ratio to the leukocytes. Red cell and platelet changes are similar to those found in the myeloid type.

Up to the present time a sufficient number of cases of monocytic leukemia has not been reported, to justify a separation into the acute and chronic forms. The leukocyte count in the reported cases varies from 600 to 320,000 per c.mm., but a majority show only moderately high counts, (25,000 to 50,000 per c.mm.). The stained smear in a typical case reveals a large number of mature monocytes with immature blast forms (monoblasts?) in the minority, although in some cases there is a preponderance of blast forms which are assumed to be monoblasts but appear identical with myeloblasts. There is no agreement in regard to the peroxidase reaction in monocytic leukemia and frequently even the autopsy fails to throw light on the question. There is no doubt, however, that regardless of where the monocyte has its origin, there does exist a rare type of leukemia which is characterized by the appearance of large numbers of cells which fit morphologically the current description of the monocyte. As in other leukemias, anemia and thrombopenia are secondary findings.

It should be remembered by the laboratory worker that infectious processes, especially in children, may simulate closely the blood picture of chronic leukemia in its early stages. Severe types of pyogenic infections with a shower of neutrophilic myelocytes may present a "leukemoid" blood picture (one resembling leukemia). Von Jaksch's anemia, certain bone marrow crises in hemolytic jaundice and sickle cell anemia may at times, show on the stained smear a blood picture of chronic myeloid leukemia. A blood smear from a

severe case of whooping cough or from infectious mononucleosis may show a lymphocytic response equal to that of lymphatic leukemia. A blood study, therefore, without the age, history and physical findings of the patient is an extremely hazardous diagnostic criterion.

As stated above, the chronic and acute leukemias may undergo an "aleukemic" or leukopenic phase, in which the circulating leukocytes drop below normal figures. It may occur spontaneously and usually presents a difficult problem when the patient is seen for the first time during the leukopenic phase. The blood picture may resemble that seen in aplastic anemia with a lowered number of leukocytes, erythrocytes, and platelets, although the red cell picture bears a closer resemblance to pernicious anemia with macrocytosis, megaloblasts, normablasts, and variation in size and shape of the erythrocytes. It is here that a careful differential count, with recognition of the immature cells, offers great aid in the diagnosis of leukemia. If the count is low, the cells are extremely scarce, so that sufficient numbers can hardly be found for study. In this event, we usually "concentrate" the white cells as follows:

Ten c.c. of blood are withdrawn from the vein, and placed in a 15 c.c. centrifuge tube containing 2 drops of 20 per cent potassium oxalate. The tube is inverted gently several times to facilitate mixing and to prevent coagulation. It is centrifuged at once at high speed, and the serum carefully decanted. Then, using a finely drawn capillary pipette, the upper surface of the red cell layer is *carefully* drawn up and small drops placed on the end of clean slides. These are then spread into blood films in the usual manner and stained with Wright's stain. In this way the few leukocytes are "concentrated" in a few smears and it is possible to secure beautiful preparations with 15 or 20 cells per field, whereas, with the ordinary smear, it is often necesary to search many fields before finding a single cell.

Laboratory Findings in the Acute Leukemias

It was thought for many years that the acute leukemias were all lymphatic in type. Since Naegeli described the occurence of myeloblastic leukemia and since the peroxidase stains have been employed, it has become evident that acute lymphoblastic leukemia is a rarity and is possibly limited to childhood. It is safer to consider all acute leukemias myeloblastic until proven to be lymphatic. It is certain that a great majority are myeloid in type and occur more frequently in young adults. The differentiation between the two is often so difficult that some authorities use the term "acute leukosis" and make no effort to distinguish between them. There are, in typical cases, some points of distinction.

Typical forms of acute myeloblastic leukemia present the following blood picture: (1) a sub-leukemic leukocyte count, usually under 50,000 per c.mm. in the early stages; (2) a preponderance of

myeloblasts and micromyeloblasts, the former closely resembling lymyphoblasts and the latter almost indistinguishable from small lymphocytes; (3) the presence of faintly granular premyelocytes and an occasional neutrophilic myelocyte with mature segmented forms in sufficient numbers to convince the observer that the associated leukoblasts are myeloblasts; (4) a peroxidase positive reaction and a positive indophenol blue synthesis in all cells but the non-granular myeloblast; (5) a severe anemia, frequently of the primary type, with macrocytosis, hyperchromia, megaloblastosis, and normoblastosis; (6) a marked thrombopenia, prolonged bleeding time and clot retraction time. When the diagnostic premyelocytes and myelocytes are absent, as is frequently the case when the patient is seen in the aleukemic phase, it is almost impossible to identify the blast forms as myeloblasts by ordinary staining methods. If one is fortunate enough to find only a few typical myelocytes the unknown blast cells may be tentatively identified by "the company they keep."

The hematologic picture in acute lymphoblastic leukemia varies only in the predominant cell type, which is the lymphoblast. There are present, in some cases, sufficient numbers of mature lymphocytes and smudge forms to render a decision in favor of lymphoblasts. The cells do not take the peroxidase stain, but a negative reaction does not rule out myeloblastic leukemia, since myeloblasts are also nongranular. The stain is diagnostic only when positive. In the aleukemic phase of lymphoblastic leukemia, again, the determination of the cell type is virtually impossible and sometimes remains so, even after autopsy.

Lymphatic leukemias, both acute and chronic, are frequently confused with infectious mononucleosis which is a benign disease of short duration, characterized by an increased number of lymphocytes, lymphoblasts and monocytes. Differentiation on morphology of the cell types cannot be done with certainty. In the latter disease, however, red cells and platelets are usually unaffected and the heterophile antibody test is of much value in differentiating between the two conditions.

Heterophile Antibody Agglutination Test

(Reproduced from the Laboratory Manual of Clinical Pathology by Parker and Garver) Technic: (Combined Methods of Davidsohn, Paul and Bunnell, and Bernstein).

1. The patient's blood serum is inactivated for 30 minutes at 55° C.
2. Dilutions of the serum are made according to the table below.
3. A 2% suspension of sheep cells, which are at least 24 hours and not over a week old, is prepared in normal saline. These cells must be washed 3 times immediately before using.

4. A method for preparation of the dilutions is given in the following table. Saline is added first beginning with tube 2. After serial dilutions are made with serum and saline, sheep cells are added. Tube 1 is not included in serial dilutions. Dilution number refers to concentration of serum in saline.

Dilution	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	Control
Tube No.	1	2	3	4	5	6	7	8	9	10	11
Saline	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Serum Inactivated	0.5	0.5 Mix & transfer 0.5 cc to tube 3	Transfer 0.5 cc to tube 4	Repeat	Repeat	Repeat	Repeat	Repeat	Repeat	Mix & discard 0.5 cc	0
Washed Sheep Cells 2%	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

5. The tubes are shaken thoroughly, incubated in a water bath at 37° C. for 1 hour and placed in the ice-box overnight (1 hour at room temperature may be substituted for the ice-box incubation, but the test should be checked after refrigeration).

6. The results are read macroscopically after gently inverting the tubes 3 times. The end point of agglutination is best determined by the low power lens of the microscope.

7. The results are reported in degrees: The agglutination is considered 3 plus when the cells remain in a single large clump; 2 plus when the clumps are definitely visible but suspended in clear fluid; 1 plus when it can be demonstrated only under the microscope.

INTERPRETATION

For some time it has been known that the blood of normal individuals contains heterophile antibodies (agglutinins and hemolysins) against red blood cells of the sheep. This phenomenon is demonstrable only in low titers. Recent investigations show that an absence of a marked increase (high dilutions of sera) of these antibodies is of diagnostic importance in clinical medicine.

The demonstration of agglutinins for sheep cells in high titers in the sera of individuals suffering with infectious mononucleosis is found with such regularity that this agglutination test is used extensively in the diagnosis of this disease.

The injection of horse serum into normal individuals will cause an elevated titer but rarely reaches the height of that found in infectious mononucleosis.

Recent research indicates that in leukemias of all forms these heterophile antibodies are either absent or are found in low titers (generally, but not necessarily below the normal level). This facilitates the differential diagnosis between certain forms of leukemia and infectious mononucleosis, which in the early stages, are often clinically and hematologically identical. Leukemia may be excluded but a low titer does not establish its existence. A titer of 1:128 in the absence of recent horse serum therapy and in the presence of suspicious symptoms almost invariably establishes the diagnosis of infectious mononucleosis. A titer of above

1:128 is considered positive for this disease even if there is a history of serum injections unless the patient has serum sickness (urticaria, etc.) at the time of examination or has only recently recovered from this condition. (See chart below). In severe cases the titer may reach 1:4000.

Heterophile Antibody Titers Under Various Circumstances (Bernstein).

	Normal			Infectious Mononucleosis				
Zone 1		Zone 2			Zone 3			
1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Leukemia	Serum Therapy								

Despite the tone of pessimism in regard to the difficulty in distinguishing between the immature cell types, there are some fine points of distinction between the myeloblast and the lymphoblast, as given in the table below. These cytologic cryteria are not infallible nor final, and a more certain method is to identify a cell "by the company it keeps."

	Lymphoblast	*Myeloblast*
Nucleus	purple, round or oval,	purple, round or oval.
Chromatin	coarse arrangement, knotted appearance, dense at the edges,	smooth, finely woven, homogeneous.
Nuclear membrane	clear cut and definite,	not perceptible.
Nucleoli	1 to 3 in number,	3 to 5 in number.
Perinuclear zone	definite,	none.
Auer bodies	none,	occasional.
Cytoplasm	deep blue,	deep blue.

Conclusions

1. Leukemia is a disease in which the total leukocyte count may range from only a few hundreds to several hundred thousand cells per cubic millimeter of blood.

2. In a leukemia with a low cell count, it is most important to

be able to recognize the immature, blast forms and not confuse them with lymphocytes, as is so often done.

3. The laboratory aids to this end should include careful morphologic study, peroxidase reactions, indophenol blue synthesis, and studies on concentrated specimens of blood.

4. The diagnosis of leukemia should never be made in a laboratory. This can be done only by the physician. A "laboratory diagnosis" of leukemia is attempted only by amateurs and never by experts.

SPIROCHAETA FROM BLOOD AND TISSUE CULTURES OF DISEASED CHICKENS

By EDWARD REDOWITZ, M.T.

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Spirochaetosis in fowl has been found to occur in many parts of the world. (1) Many specific names have been suggested for the different strains of spirochaetes that have been found in fowls in tropical and subtropical countries. (2) SPIROCHAETA ANSERINA and SPIROCHAETA GALLINARUM (3) (now considered as the same species) have been extensively studied and described as the etiological agents in fowl spirochaetosis. However, the literature gives no account of these organisms as having been found in the northern parts of the United States.

Harris (4) in 1930 reported to have found spirochaetes in the intestinal tract of healthy and diseased chickens. She was able to cultivate them in vitro after the method of Noguchi (5) using tissue, agar, ascitic fluid medium under oil. These spirochaetes were never found in blood smears and only in one instance were they detected in the lungs. Three distinct types were observed and designated as TREPONEMA CAECI—gallorum, SPIRONEMA CAECI—gallorum and FUSISPIROCHAETA CAECI—gallorum. All three were evidently non-pathogenic.

In the fall of 1934 a poultry farmer from the state of New Jersey brought to the laboratory three very sick chickens. The one having the most marked symptoms showed emaciation, remained inactive and did not eat. The other two birds were also emaciated, but they appeared more active and took food. Their combs were dry and pale.

The farmer has been in the poultry business for over fifteen years. His main income has been from the sale of eggs. He always hatched and raised his own chickens from a stock of White Leghorns.

The hennery consists of two large poultry houses with concrete floors located on a tract of land three and one-half acres in size. Water is obtained from two wells on the premises.

About five years ago chicken-pox appeared and for three successive years all the grown fowl were vaccinated against this disease. However, in 1934 this farmer suffered a tremendous loss through a diminished supply of eggs and heavy mortality among

the birds. Out of a flock of 3,000 yearling pullets 1,300 had died up to December first, 1934.

The first symptoms he noticed were dryness and paleness of the comb. Gradually the birds would eat less, stop laying, lose weight, show general weakness and die in five to eight weeks. The droppings of these birds were normal in appearance.

At first the farmer suspected the well-water. Bacteriological examination of the water and the feces of these chickens were negative for pathogenic organisms. It was then suggested to the farmer that some of the sick birds be brought to the laboratory for study.

On December 6, 1934, the birds were received. CHICKEN NO. 1 (having the most severe symptoms) was bled from the jugular vein, 5c.c. of blood obtained and placed in 100c.c. of 1.0% dextrose veal broth for culture. The bird was then immediately killed. The only marked pathology was a very much enlarged liver, which was very soft, friable and of grayish appearance. The odor of this chicken was very strong and fetid.

Cultures were made of the heart muscle, lungs, spleen, liver and kidneys by removing a section of tissue and dropping it into a tube containing 5c.c. of Hitchens semi-solid agar broth.

This medium is prepared by adding 0.1% agar to the finished veal broth containing 1.0% Bacto peptone and 0.5% sodium chloride. After the agar is melted in the Arnold sterilizer the reaction is adjusted to pH 7.8 heated again for five minutes, filtered through paper and autoclaved at 10 pounds pressure for 20 minutes.

All the cultures were incubated at 37°C. After several days of incubation, a very faint cloudiness had developed near the surface of all the tissue cultures, with the exception of the liver tube which showed no change. On the fourth day smears made and stained by Gram's method revealed spirochaetes and free coccoid forms. The spirochaetes were Gram negative but the coccoid forms retained the gentian violet stain. The first impression was that a mixed culture existed, but in subsequent transplants the coccoid forms disappeared and a pure culture of the spirochaetes was observed.

CHICKEN NO. II was kept for observation. On December 7th, blood smears were made which showed a slight degree of anemia. On December 18th hemoglobin was 45% (Dare) erythrocytes 1,520,000. Differential smears revealed no pathology but did show evidence of anemia. Blood culture was made in semi-solid agar broth; this culture developed the same type of growth in six days as the blood culture of chicken NO. I. Smears made of the

film showed numerous spirochaetes. The bird died over the week-end 18 days after it was received at the laboratory.

Autopsy revealed a slightly enlarged reddish-brown liver, no other lesions observed. This bird also had a fetid odor.

Cultures made from sections of the internal organs upon incubation showed only contaminating organisms due to the bird being dead for some time.

CHICKEN NO. III was also held for observation. On the following day after being received, blood smears appeared normal, hemoglobin 76%, erythrocytes 2,010,000, blood sugar 256 mgms.

December 18th differential smears revealed no pathology. January 4th, 1935, the bird appeared very ill, lying on its side in the cage. It was killed and the tissues were cultured in the same manner as for CHICKEN NO. I. After ten days of incubation the culture of the heart tissue alone showed growth. Smears from this culture showed Gram-positive cocci in pairs. No growth was obtained in subcultures in serum-dextrose broth and blood agar plate. The autopsy did not reveal any gross pathology. This chicken did not have as much loss of flesh as the other two nor the fetid odor.

The spirochaetes isolated from chickens No. I and No. II are identical and have been kept alive in cultures up to this time; passing through the 102nd generation. Transplants are made every four to five days in semi-solid agar broth. A loopful of the growth is rubbed on the side of the tube so that it remains on the surface of the medium. The growth occurs on or near the surface. If the tube is incubated in a slanting position the growth spreads over the entire surface of the medium in four to five days, thus, indicating that its growth requirements are aerobic. In young cultures very short forms were observed, with only two or three curves, later as the maximum growth is reached (5 to 6 days) very long forms having as many as fifty curves were seen. Noguchi (5) made similar observations in cultures of S. DUTTONI and S. OBERMEIERI. They grew more vigorously if a piece of fresh rabbit liver or kidney was added to the medium. The cultures, young or old, had no perceptible odor at any time.

The formation of coccoid bodies attached to the spirochaetes described by Noguchi (6) in cultures of SPIROCHAETA GALLINARUM were never observed. However, smears from young as well as old cultures invariably showed some small coccoid bodies independent of the spirochaetes. In some smears made from five day old cultures short spirochaetes were seen that had peculiar swellings at one end. The end including a curve or two would be

considerably thickened. Then again, on a long spirochaete near the end a spore-like rounded enlargement could be noted, possibly degeneration taking place within the cell. Due to the extreme morphological variation in cultures no emphasis can be placed on the morphology of this spirochaete as an aid to its classification.

In cultures examined by dark-ground illumination or in a hanging drop, the spirochaetes were clumped, although some actively motile free forms could always be seen. Their movement is very rapid and they are able to travel in either direction. The ability to move in either direction is the characteristic motion of spirochaetes described by Hindle (2).

They stain well by carbol fuchsin, Wright's and Giemsa's method. Smears dried in the air and fixed by gentle heating were quite satisfactory. In smears made from cultures five days old, some long forms were observed with a very thin portion in the center, and also chains of three or four that appeared as if division had just taken place. This suggests that transverse division is the method of multiplication.

The spirochaetes were never found in blood smears nor in the smears made of tissues from which they were isolated in culture. This is probably due to their peculiar life cycle. It has been reported that some spirochaetes at certain stages of development are so small that they readily pass through porcelain filters.

None of the blood smears revealed the intracorpuscular granules observed by Balfour (7) and others in the blood cells of fowl suffering from spirochaetosis. Attempts to infect young chicks, hens, rats and guinea pigs have been unsuccessful so far.

No satisfactory agreement has been reached on the classification of the various spirochaetes. However, Hindle (2) and Wenyon (1) agree that the correct designation for all fowl spirochaetes including those of other domestic birds such as ducks and geese is SPIROCHAETA ANSERINA.

According to Bergey's (8) latest classification, fowl spirochaetes are placed in the genus BORRELIA. As far as could be ascertained from the literature, the spirochaete under study is the first aerobically cultivated fowl spirochaete described in the United States. Whether or not this would place it in a distinct species is a matter for consideration. Its proper descriptive designation under Bergey's genus for fowl spirochaetes would be BORRELIA PULLORUM.

The necessity for the further study of this organism as to its source, pathogenicity, etc., is apparent.

Plate I—From a three day culture, all very short spirochaetes. X2150. Fixed by heat and stained for one hour with Giemsa's

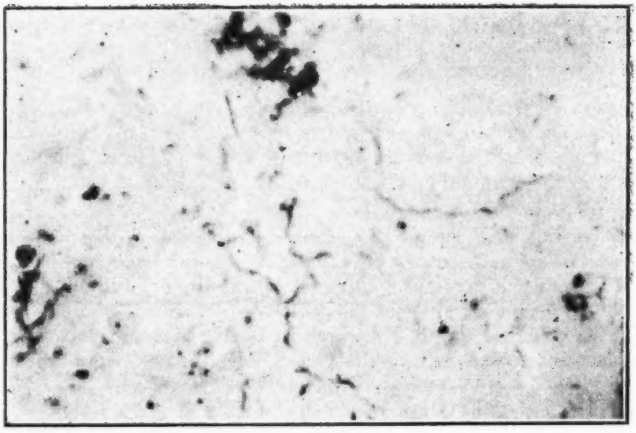

Plate II—From a five day culture, showing longer and more slender forms. X2150. Fixed by heat and stained for one hour with Giemsa's.

Summary

A spirochaete isolated from blood and tissues of diseased chickens has been successfully cultivated aerobically, in veal broth containing 0.1% agar, having a final reaction of pH 7.6.

BORRELIA PULLORUM as a descriptive name is suggested. The report is submitted with the hope that the method described in isolating and cultivating the organism, may be helpful in the study of spirochaetosis in fowl.

I wish to express my appreciation to Dr. Claude P. Brown for his guidance and encouragement in this work; to Dr. David H. Bergey for suggestions on the classification; and to Dr. E. L. Stubbs of the Veterinary School of the University of Pennsylvania, whose help in interpreting the blood smears of chickens has been very valuable.

References

1. Wenyon, C. M.: Protozoology, William Wood and Co., New York, V2—1261-1267, 1926.
2. Hindle, Edward: A System of Bacteriology in Relation to Medicine, His Majesty's Stationery Office, London—V8 pp. 101-184, 1931.
3. Hindle, Edward: On the Life-Cycle of Spirochaeta Gallinarum. Parasitology V4: 463-477, 1911.
4. Harris, Minne, B. K.: A Study of Spirochaetosis in Chickens with special Reference to those of the Intestinal Tract. The Amer. Jour. of Hyg.—V12 No. 3, 537, 1930.
5. Noguchi, Hideyo: The Pure Cultivation of S. Duttoni, S. Kochi, S. Obermeieri, and S. Novyi. Jour. Exp. Med.—V16, 199-210, 1912.
6. Noguchi, Hideyo: Cultivation of Spirochaeta Gallinarum, Jour. Exp. Med. V16, 620-628, 1912.
7. Balfour, A.: A Peculiar Blood Condition, Probably Parasitic in Sudanese Fowls. Jour. of Trop. Med. and Hyg., V10, 153, 1907.
8. Bergey, David H.: Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Co., Baltimore, pp. 624-627, 1934.

PATHOGENIC FUNGI

STUDIES FROM TWO FATAL CASES

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Present literature abounds with material on pathogenic fungi. Since this is essentially a study of budding fungi isolated from sputum of two patients who died, I shall consider only those fungi associated with pulmonary pathology.

Historical

The first studies of fungous disease in man began about 1840 when Berg saw the budding fungi in disease of the mouth and throat. Robin in 1847 studied the organism of thrush from membraneous patches in the throat. He described filamentous forms with little spores forming on the sides. He named it Oidium Albicans, believing it to be the same as the fungus of grapevine mildew called Oidium by Link.

Link (1) in 1809 had described his fungus as segmented filaments, producing at their extremities short cells, very often rounded. The cells united end to end rosary like, and detached themselves when mature. These spores produced more filaments. Vuillemin in 1898 reported asci in the cells and called the organism Endomyces Albicans. Since pure culture methods have been perfected the thrush fungus has been proved not to have asci and has therefore been called Monilia albicans.

In 1884 Busse in Germany, first reported a case of generalized fatal yeast infection, caused by a budding fungus. He called the infection Blastomycosis. The patient lived thirteen months after the initial infection. Beginning with a tibial abscess it extended through the lymph nodes involving the ulna and one rib in abscesses. Later the lungs, liver, kidney, and spleen were affected. Buschke, studying this case, isolated budding yeasts from the blood stream eight weeks before death.

Gilchrist, in 1896 (1) observed budding yeasts causing dermatitis. He described the cells as spherical, 10 to 17 micra in diameter, having a homogenous double contoured capsule, the cytoplasm showing fine and course granules and frequently vacuoles. In the

tissues he found only budding forms. The dermatitis sometimes preceded a generalized infection that became fatal.

Rickets (1) in 1902 summarized in an exhaustive monograph, the study of those early cases first noted in the Chicago region.

In 1912 Castellani (2) began to classify the fungi found in tropical bronchomycosis. He recognized: 1, Endomyces, round to oval cells sometimes with mycelium, showing on sugar agar round thick creamy white colonies; 2, Saccharomyces, round cells budding only in old cultures; 3, the molds; and 4, the actinomyces group. He has since (3) extended his classification, basing it mainly on sugar fermentations and gelatin liquifaction.

Steinfield (4) divided his classification of throat fungi into Endomyces, those forming endospores; Monilia, those showing both budding forms and filaments, and Cryptococcus, those having little or no mycelium.

Buschke's term, Blastomyces, has become common as a medical term for all budding fungi. While deploring this inaccuracy, Ota, (5) 1924, divided the Blastomyces into Saccharomyces, Cryptococcus and Monilia. His studies are based on morphology, growth on beer wort agar and liquid, of cultures at varying periods, on spore formation and on sugar reactions.

Castellani's early studies stressed the possibility of the yeast-like fungi causing a condition similar to pulmonary tuberculosis. Since then the fungi have been incriminated in bronchitis, pneumonia, and bronchial asthma as well.

Tanner and Dack (1924) (6) studied 22 strains of yeast-like fungi from sore throats. Only one was not pathogenic for animals. They injected non-pathogenic yeasts into animals with only one apparent reaction. Tanner, Lampert and Lampert (7) found yeast-like fungi in 10 per cent of 1000 normal throats. Many of these were pathogenic for white mice.

Methods of Detection and Isolation

When tubercle bacilli have not been found in several successive sputum specimens, a specimen is poured into a petri dish and diluted with sterile saline. Over a dark background search is made for tiny firm white masses. They may be removed with a capillary pipette and crushed between slide and coverslip or washed through saline and cultured.

The budding fungi are present mainly in these tiny granules. They appear as masses of short filaments, 0.8 x 10 micra, sometimes branched or budded; sometimes there are round budded forms apart from the filaments.

The presence of fungi having been demonstrated in sputum, the patient is asked to wash his mouth thoroughly with sterile normal saline and after that to cough up material from deep air passages into a sterile petri dish. From this the white flecks are selected, washed through more saline and planted in dextrose broth and on malt agar.

Growth in dextrose broth was slow because of contaminating streptococci. For isolation after the budding fungi were started in the broth, we used Tanner's lactic acid malt agar (7) and later, Hill's (8) near beer agar. The malt agar is prepared as follows:

1000 cc. distilled water
5 gm. Bacto peptone
3 gm. Liebig's meat extract
15 gm. Bacto malt
Autoclave, and under aseptic technic, tube in 5 cc. quantities.
When cool, but not solid, add 0.2 cc. sterile 5% lactic acid solution to each tube and shake vigorously before slanting.

The near beer agar uses:

Contents of 1 pint bottle near beer
Make up to 900 cc. with tap-water
Add 14 g. agar shreds. Dissolve by boiling.
Filter; autoclave in 100 cc. portions.

Before pouring plates add to each 100 cc. melted agar 4 cc. sterile lactic acid.

Carrot slants were also used, as well as Sabouraud's media. The latter contains 1 per cent peptone, 4 per cent crude maltose and 1½ per cent agar. However, we found it not so good for isolation work as the bacteria were only partially retarded. After the organism had been isolated in pure culture they grew readily on all the generally used media. Growth was most luxuriant on dextrose agar so stock cultures were kept on that.

Methods of Study

In attempting to identify budding fungi we have studied the microscopic appearance of young and old cultures, the macroscopic appearance of tubed cultures and of giant colonies and the biochemical reactions to sugar broth and to milk.

To study pathogenicity we innoculated a guinea pig and two rabbits. Following Castellani's (9) method, one rabbit was innoculated intravenously using ¼ cc. of a heavy suspension in saline from a 24-hour growth on plain agar slant, while the other rabbit was innoculated with a similar dose into the left lung. At autopsy, cul-

tures were made from all organs and sections were fixed and stained with hematoxylin-eosin.

Sugar broths were made by adding 10 per cent sterile sugar solutions in 1-10 proportion to sterile broth in tubes.

General Procedures

CASE 1. This patient, a farmer, ill for six months after removal of an acute appendix, was expectorating much foul-smelling, blood streaked sputum. Tubercle bacilli were not found, but in the carefully collected specimens there were seen many of the small white flecks containing the filamentous masses. A first culture on blood agar showed a few long threads like a mold, with terminal swelling. Later on there were many budded forms in the sputum. When the broth cultures were transferred to malt agar the growth was of budded yeasts with some elongated cells.

Cultures of pus from beneath the scab of the appendiceal incision were sterile. A blood culture showed no growth after a week. When an exploratory laparatomy was performed a hard nodular piece of liver was excised and sent to the laboratory. One portion of this, crushed in 20 per cent Sodium Hydroxide between slide and coverslip showed filaments with terminal swelling. The second portion, ground with sterile sand and planted in dextrose broth remained sterile after three months. The third portion, on section showed an infectious granuloma, neither tuberculous nor luetic.

Drainage from this incision showed no budding fungi and cultures showed streptococci. After 7 to 10 days the budded forms appeared in the dextrose broth. After 3 days more these were successfully transplanted to malt agar and a pure culture was obtained similar to that from the sputum. Another blood culture was made and left indefinitely at room temperature not to be disturbed until signs of growth appeared.

Cultures from urine and stools showed budding forms in a mass of mycelium, but not before 3 weeks' standing. After 25 days there was a faint black ring at the surface of the blood culture. Microscopically there was a pure culture of much larger yeasts than any of the others already isolated, and some mycelium.

Systematic Study

1. MACROSCOPIC APPEARANCE

A. All the strains except that from blood were creamy white, growing readily at room or incubator temperature on dextrose or

near beer agar. Giant colonies in three weeks reached 2 inches diameter. Scum developed on the surface of liquid media, later settling to the bottom.

B. The blood organism on dextrose or near beer agar was at first jet black, usually dull, sometimes glistening. In broth there was black scum at the surface. Later the transfers varied, part black, part white, sometimes brownish. The giant colony in 5 weeks at room temperature covered a 4-inch petri dish. A short cottony tuft of hyphae extended above the surface.

2. Microscopic Appearance of Cells

A. Pus and other strains. After 3 to 15 days on near beer agar, cells were round to oval, 3 to 6 micra, occasionally elongated, usually granular. Rudimentary mycelium in form of 2 to 3 long cells in series, with usually a rounded cell at one end. Often several cells grouped around one or two larger cells.

B. Black strain. After 3 days on near beer agar, cells mostly oval, in general larger than those above, granular, containing one or two vacuoles. Buds at the end, at the side or both. Elongated cells growing out from the end, contained vacuoles. Large round cells, 7 to 8 micra, sometimes slightly oval were darker, showed no vacuole and had their granules arranged to form tiny rings.

After 15 to 30 days there were: oval cells 4 to 8 micra, single, in pairs or in small groups; many large dark cells, long cells, often containing granules, arranged in series forming a branched mycelium; sometimes in the middle of a chain 1 to 10 very dark cells that might bud laterally; many short chains of 2 to 10 cells of dark mycelium.

After 6 weeks more cells showed fat globules. These were mostly the large round to oval cells, some with thickened walls. There remained the dark cells and the mycelium as before. On Gorodkowa's agar after 3 weeks no ascospores were found.

3. Biochemical Reactions

Within three months of isolation all five strains fermented dextrose, maltose, levulose and lactose. After ten months when there was opportunity for more extended study there was slight reaction. The pus strain within one month after passage through a guinea pig gave vigorous fermentation with gas and acid in dextrose, levulose, mannose, maltose, galactose, saccharose, lactose, innulin, raffinose, dextrin, silicin, arabinose, melizitose, dulcite. It gave acid and curd in milk, darkened lead acetate paper, reduced nitrates.

4. PATHOGENICITY

The strain from pus was pathogenic for the guinea pig. Cultures of budded yeasts were recovered from lung, liver, kidney, spleen and from fluid in the abdominal cavity. In direct examination of this fluid there were no budded yeasts. There were round cells though, each with a long process going out from it. These resembled the forms noted in the fresh preparation from human liver.

Sections of lung, kidney and spleen showed abscesses of varying size. Often among many polymorphonuclear leucocytes there were seen one or two round unstained cells, double contoured, having a very long budding process. Thread forms were numerous throughout portions of the lung and kidney. These resembled thread forms found in sputum.

CASE 2. This organism was isolated from sputum of a woman having lung and pleural involvment. In several carefully collected sputum specimens acid-fast bacilli were never found but there were budded yeasts and filaments as in Case 1. This patient died 6 weeks after these findings.

Pleural fluid showed no acid-fast bacilli and cultures into deep tubes of dextrose brain broth showed no clouding in 48 hours. But after 14 days at 37° C., there was a granular sediment at the bottom. This showed elongated cells resembling a monilia. This patient was not in the hospital. One urine and one blood specimen were submitted for culture but no fungi were demonstrated.

Systematic Study

1. MACROSCOPIC APPEARANCE

A. Sputum strain. Luxuriant creamy white growth on near beer agar at room temperature or 37° C. In 3 weeks the giant colony reached a diameter of 4 to 5 cm. with secondary colonies.

B. Fluid Strain. On dextrose agar and on near beer agar, growth was slow at room or incubator temperature. It was greyish white, granular, tenacious. With age the surface of the culture became dry and dusty. The giant colony in 3 weeks was 2 to 3 cm. in diameter, and appeared wrinkled.

2. MICROSCOPIC APPEARANCE

A. Sputum strain. Round to oval cells, some elongated, some budded. Mycelium was not often present in the young cultures although the short threads had been numerous in fresh sputum specimens. No ascospores were found after 3 weeks on Gorodkowa's media.

B. Fluid strain. Round and oval budding forms present but most cells were long, often tapered at the end. Sometimes these narrow filaments extended half way across the high power microscopic field. Many long cells budded at one end. Cells contained granules and 2 to 4 vacuoles. No ascospores were found.

3. Biochemical Reactions

A. Sputum strain: Gas and acid in dextrose, levulose, mannose, maltose, galactose. No reaction in xylose, dextrin, salicin, arabinose, dulcite, melizitose, raffinose, Lactose, salicin, dulcite turned alkaline.

B. Fluid strain: Slight acidity in mannose. It turned all the other sugars alkaline, producing no gas.

4. Pathogenicity

A. Sputum strain. About one week after injection into a guinea pig the animal grew listless, steadily lost weight, and the inguinal glands enlarged. Death occurred in 3 weeks but the animal was disposed of before it was examined.

Two weeks after isolation one rabbit was innoculated intravenously, and another was innoculated in the left lung. The latter showed the greatest reaction. On the 3rd day its breathing became quick and labored. On the 5th day it died. Cultures for fungi were positive from lung, kidney, spleen, heart, urinary bladder and from an embryo. Cultures from lung and heart showed mycelial threads; the others showed only budded forms. The lung strain gave the same sugar reactions as the patient's sputum strain. Cells appeared the same after several transfers.

Tissue sections showed the greatest change in lung and kidney. Thread forms were evident particularly in the distended alveoli of the lung. These organisms were about ten times their breadth in length. They did not stain with hematoxylin-eosin. They appeared somewhat yellow tinged and refractile. No budded yeasts were seen.

The rabbit injected in the ear vein sat in stupor for a week or two then began to frisk about. After 6 months it was sacrificed. No tissue lesions were found and all cultures were negative for budding fungi.

B. Fluid strain. Dry, granular and tenacious, this could not be prepared in even suspension. However, ¼ cc. of a granular suspension was injected subtesticularly into a guinea pig after the

method of Long (10). After 4 weeks the pig began to lose weight. It died at the end of 7 weeks.

At autopsy all lymph nodes were found to be enlarged and showed long threads in caseous material. Cultures from these and from lung, spleen and testis grew slowly in dextrose broth and in near beer broth but not on solid media. After 10 days at room temperature the liquid was still clear but there was a slightly granular sediment at the bottom of the tubes. It showed the long slender cells often with each end tapering, and vacuoles within. Smears from fluid in the peritoneal cavity showed narrow elongated cells. The organism was observed in lung tissue where the reaction resembled that of the sputum strain in the rabbit lung.

Summary

Both of these fungi accorded closely with Ota's description of the Monilia: (1) no asci found; (2) budded yeast forms present in young cultures, with mycelium increasing after a few days; (3) cultures of a month or so still showing mycelium, and the round isolated cells increased greatly in size, having a thicker capsule, and containing more granules and fat globules; and (4) producing acid and gas in dextrose, levulose, mannose, and galactose.

Both these fungi were pathogenic for the guinea pig. The second fungus was also pathogenic for the rabbit.

Conclusion

We believed these two generalized infections were due to Monilia albicans, with variant strains in pleural fluid or blood.

This study was carried on under guidance of Prof. F. W. Tanner of the University of Illinois department of Bacteriology.

BIBLIOGRAPHY

1. Ricketts, H. T.: Oidiomycosis (Blastomycosis) of the Skin and Its Fungi. J. Med. Res. 6: 377. 1902.
2. Castellani, A.: Observations on the Fungi Found in Tropical Bronchomycosis, Lancet 1:13 (Jan.) 1912.
3. Castellani, A.: Bronchomycosis, Milit. Surg. 57:113 (Aug.) 1925.
4. Steinfield, E.: A Study of Yeasts Found in the Sputum of Patients with Asthma and Chronic Bronchitis, J. Lab & Clin. Med. 8:744 (Aug.) 1923.
5. Ota, M.: Das Systematische Problem der pathogenen, asporogenen Hefen und der ihnen nahestenden Pilze. Dermatol. Wchnschr., 78:9, 1924. Ota, M.: Essai de Classification des Blastomycetes pathogenes. Annal d. Parasitol., 11:34, 1924.
6. Tanner, F. W. and Dack, G. M.: A Study of Yeasts from Sore Throats, Centralbl. f. Bacteriol. 91:282, 1924.
7. Tanner, F. W., Lampert, E. N., and Lampert, M.: On the Presence of Fungi in Normal Throats, Ibid. 103:94, 1927.
8. Hill, G. A.: The Isolation of Yeasts and Molds, J. Lab. & Clin. Med. 13:765 (May) 1928.
9. Castellani, A.: Fungi and Fungous Diseases. Chicago, Illinois, American Medical Association, 123. 1928.
10. Long, E.: Hypersensitiveness, J. A. M. A., 87, 1441.

THE CYTOLOGY OF SPINAL FLUID

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From a pathological standpoint the study of the cytology of spinal fluid elicits more information regarding the probable diagnosis in central nervous system disorders than any other laboratory test. It is of importance to obtain the information that the spinal fluid does not contain an increased number of cells. By such a report the possibility of a number of diseases may be eliminated.

In many conditions the number of cells is so slightly increased it becomes a controversy whether there is an increase or not. Whereas some consider 10 cells as normal, others consider above 5 as pathological. In conditions such as tuberculosis, encephalitis, lues, and certain stages of poliomyelitis there is an increase in the number of cells of the type normally found, namely lymphocytes (or monocytes as they are sometimes called). In the acute infectious diseases such as the pneumococcic, streptococcic, meningococcic and influenzal meningitis the polynuclear leukocytes predominate and the number of cells found per cubic millimeter may be in the thousands.

Not only the number of cells present but also the differential count of the cells is very important. It is preferable to do the cell count as soon after the spinal fluid is drawn as possible, in order to get a uniform distribution of them. After standing, fibrin may form and the cells be caught in the meshes of it and the cell count be unreliable.

The method employed in our laboratory is as follows: The diluting fluid consists of 30% acetic acid with Methylene Blue added. A white cell pipette is used. The diluting fluid is drawn up to the "I" mark and the spinal fluid up to the "II" mark. The spinal fluid is shaken well just before it is drawn up. An ordinary hemacytometer or Fuchs Rosenthal chamber is used and the proper correction made for dilution. The presence of more than five cells is considered pathological. The remainder of the fluid is centrifuged and the supernatant fluid used for the determination of globulin and sugar. The sediment is placed on two glass slides and smears allowed to dry in the air. One smear is stained with Wright's stain, the other with Gram's stain.

When there is an increase in the number of polynuclear cells and bacteria are seen, the diagnosis may be made immediately. But

in certain other diseases there may be an increased number of lymphocytes. It is concerning one type of such a disease that I wish to comment here.

In encephalitis lethargica the number of cells found may vary from the normal number of four or five to three or four hundred with the lymphocytes predominating up to 99 per cent. After a physical and neurological examination and a carefully counted spinal fluid, the diagnosis of acute encephalitis is often made. A number of patients suffering with this disease were treated with X-ray. The spinal fluid was examined at various intervals to follow the progress of the disease until recovery. Some experimental work was carried on to prove that the X-rays were beneficial in this disease. A series of mice were inoculated intranasally with the St. Louis strain of encephalitis virus and then treated with the Roentgen Rays. Without any treatment, the mice so inoculated died within six or seven days, having shown symptoms from six to twenty-four hours. The mice given the X-ray treatments either lived longer with symptoms or recovered completely.[1] The fact that the treatment must be instituted early makes it imperative that the cytology of the spinal fluid be reported as soon as possible.

[1] Goldberg, S. A.; Brodie, M. and Stanley, P. Proc. Soc. Exper. Biol. Med. 32, 587.

EDITORIALS

THE MEDICAL TECHNOLOGIST AND NATIONAL HEALTH EMERGENCIES

The clinical laboratory worker occupies an inconspicuous place in the drama of life. His or her work is necessarily "back stage," yet the careful performance of these duties are essential for the success of the "production." The results of the laboratory test must supplement the clinical findings in order that the patient may receive the full benefit of existing knowledge in diagnosis and treatment.

In the control of epidemics of communicable diseases, the work of the laboratory technician is indispensable. Sources of infection in water and milk, as well as other foods, the detection of "carriers" of contagion, the diagnosis of existing infection often depend upon the work of the technicians.

The recent floods in certain sections of the United States were vividly portrayed by the press. Those who lived in these areas and, to some extent, those who visited the scenes of devastation appreciate more fully the property damage, human suffering, sickness, and loss of life than do the majority of those who gained information merely by reading the newspapers. However, through the efforts of the Red Cross and other humanitarian organizations, all may appreciate the important work which devolved upon the doctors, sanitarians, public health workers, and their associates.

The work of the Laboratory Technician in this disaster deserves special mention. Numerous laboratories were called upon to respond to the emergency in order to control the health situation and check the spread of disease. Many technicians in hospital and public health laboratories willingly worked overtime and cheerfully sacrificed personal convenience in assisting physicians and public health workers in their attempts to cope with the conditions. In such an emergency, various diagnostic tests, clinical bacteriological procedures, and laboratory checks are of the utmost importance. Other technicians were busily engaged in preparing vaccines and antiserums urgently needed in the stricken areas.

This is but one instance of the important work which is being done by the Clinical Laboratory. The satisfaction which attends the successful performance of service such as this surpasses any material recompense. The technician has cause to regard his calling with pride.

THE FOURTH ANNUAL CONVENTION OF THE AMERICAN SOCIETY OF CLINICAL LABORATORY TECHNICIANS

Those who were fortunate enough to have attended the Third Annual Convention of the American Society of Clinical Laboratory Technicians held last year at Atlantic City, realize that in reality it was the epoch making event of the Society. At that time, the full meaning of the organization was exemplified in the dignity with which the Convention was conducted and in the type of program given by men whose reputations are widely recognized. It marked the beginning of a year wherein the establishment of a Society for elevating the status of qualified laboratory workers became a fact with worthwhile recognition.

With the advent of the Fourth Annual Convention to be held at the Elms Hotel, Excelsior Springs, Missouri, May 11 through 13; those attending will note a definite increase in members and an array of technical procedures outlined in papers given, attesting to the rising interest in the Society.

Excelsior Springs has much to offer. The scenic beauty is beyond comparison and as a resort it numbers the most prominent citizens as its guests. A spacious auditorium within the Elms Hotel will accommodate all the programs of the Convention. Aside from the advantages of attending, the possibilities of a most enjoyable vacation are unlimited.

The American Medical Association and affiliated groups will be among the first to enjoy Kansas City's magnificent new Municipal Auditorium. Transportation will be provided from the Elms Hotel to the Auditorium, making it convenient for members to attend the A. M. A. Exhibit.

Greater Kansas City, including Excelsior Springs, a combined agricultural and industrial metropolis, is unrivaled in beauty, and is situated in the heart of the United States, geographically. There are also many places of historical interest in and around the city.

In the interest of the Society, it is the wish of the Official Board that all who possibly can, be present for the entire Convention. Valuable material will be given and a deeper appreciation of the meaning to the individual laboratory technician for the profession he or she has chosen, will result. Ever striving with a unity of purpose—that of sharing experience and findings—the Society of Clinical Laboratory Technicians will grow in proportion to the benefit derived therefrom by those who are members. May this Convention mark another step forward.

NEWS AND ANNOUNCEMENTS

BOARD OF REGISTRY
OF A. S. C. P.

As this issue goes to press, preparations are being made for holding the Sixth Semi-Annual examination which will take place in the latter part of April. From present indications a large class will face the crucial test before one of the hundred leading clinical pathologists appointed as examiners by the Registry.

The Registry has launched an energetic publicity campaign to make its usefulness known to the medical and hospital professions through the channels of the columns of medical and hospital journals. Physicians and administrators are thus acquainted with the advantages of having only qualified laboratory personnel in their respective institutions and offices.

The Board of Registry will hold a very important session at the next convention of the American Society of Clinical Pathologists which is to take place in Kansas City, May 8, 9 and 10. Questions of vital interest to technicians and training schools will be taken up, such as improvement and elevation of standards, a possible reclassification of technicians and the elimination of inadequate and commercial training schools.

The office of the Registry is exceedingly busy as a result of the increasing demands on its services and the enormous correspondence. Additions have been made in the clerical personnel and office space with new desks, typewriters, files and equipment. A more detailed description with photographs will be given in a future issue of this journal. It is also intended to give our registrants, members of the A. S. C. L. T. and readers of this journal a more intimate account of the daily routine of the Registry.

The Registry is preparing for a large delegation of visitors from the convention of technicians, many of whom are desirous of utilizing this opportunity to visit "Colorful Colorado" and the Rocky Mountains. Such a journey would not be complete without a call at the Registry office where a warm welcome will be given them. It is only a comparatively short distance, as measured out west, from Kansas City to Denver. Do not fail to include a visit to the Registry in your itinerary.

NATIONAL

To the Officers and Members of the American Society of Clinical Laboratory Technicians,

Greetings:

On the eve of our fourth annual convention, to be held in Excelsior Springs, Missouri, I wish to express my sincere thanks for the hearty and loyal co-operation of our officers and committee members throughout the year.

To these, and to every member of our Society, whose support has made it possible for us to carry on, I desire to extend an invitation to be present, to participate in the duties, and to share in the privileges of the assembly.

And to all, as well as to the Doctors who have so generously consented to take part in our program, I bid you welcome.

ROBERT C. JENKINS, *President.*

The State of Washington has reported an annual increase in the number of cases of Small-pox during the past three years. For the first nine months of 1935 the total number reached 1,014, while in 1934 there were 580, and in 1933 only 288.

It being a custom in France to name streets after distinguished citizens, and more recently after those in the medical profession who have rendered noteworthy services, Emile Roux, the former director of the Pasteur Institute, has now received this memorial.

The Chinese Medical Association recently celebrated its one hundredth anniversary of scientific medicine in China. Dr. Peter Parker, a medical missionary, first introduced western medicine into that country.

New York Times has reported that the Nobel Prize in Medicine for 1935 has been awarded to Dr. Hans Spemann, professor of zoology at the University of Freiburg in Breisgau, in recognition of his research on embryonic evolution.

At a meeting of the Academy of Medicine in Paris, attention was called to the necessity of preventative means to protect against undulant fever. Experiments are being carried out toward attaining a preventive vaccination in human beings.

BOOK REVIEW

A TEXTBOOK OF LABORATORY DIAGNOSIS with Clinical Applications for Practitioners and Students of Medicine. By Edwin E. Osgood, M.A., M.D., Assistant Professor of Medicine and Biochemistry, Director of Laboratories, University of Oregon, School of Medicine, Portland, Oregon. Second Edition, 1935. P. Blakiston's Sons & Co., Philadelphia, publishers. Pp. 585, washable cloth, Price $6.00.

This is essentially a book for medical students and practitioners but the technician will find it an original, up-to-date and practical addition to the laboratory library.

The book has a rather unique plan. The first part is devoted to pathological changes in various organ-systems and in their evidences as determined by the laboratory. There is an excellent correlation of the clinical and laboratory manifestations, and the indications for laboratory procedures in various disorders. The limitations of the laboratory in making diagnosis or predicting the course of disease are aptly pointed out. The laboratory staff would be gratified if Part I were compulsory reading for internes and residents, since they appear almost as liable as the laity to expect the impossible of the laboratory in this direction.

Part II deals with laboratory methods. This is complete but does not claim to be exhaustive. It contains many of the newer methods, including the Paul and Bunnell test for heterophile antibodies, a method for serum bromides, the congo red test for amyloidosis and the Ascheim-Zondek test and Friedman modification. References to the original literature are given at the foot of each page. The index is very complete and readily usable.

The author speaks from wide practical experience and this is very evident when the book is studied. In the preface he states that over 300,000 tests have been run in his laboratory since the first edition of the book appeared. Only the practical tests which can be applied in the ordinary clinical laboratory economically and expeditiously are suggested or outlined. Dr. Osgood has escaped the pedagogical and impractical research outlook which usually spoils the "school" book for the clinician. Nevertheless, the book is scholarly and shows the imprint of original endeavor. His uniform system of hematological study is especially to be commended.

The style is facile and the book is good reading. For instance, in his chapter on "General Considerations" when he is introducing

the second year medical student to the laboratory, he gives him some fatherly advice and makes some comments which should become classic. I quote one such, which has an especial appeal for the technician. "Do not expect a technician to be able to do better laboratory work than the physician under whose supervision she is working. She may, and often does do better work than the physician employing her could do, but if he cannot do the work himself, he is not competent to decide whether her work is satisfactory and therefore cannot be certain as to its reliability." This has long been a contention of the reviewer and while it is addressed primarily to our directors, it might be well for the technician to remember it. It's a good idea to choose a good director.

The book is well printed and bound in new washable cloth. The illustrations and tables are excellent and numerous. There are ten color plates.

ACCURATE BLOOD COUNTS with
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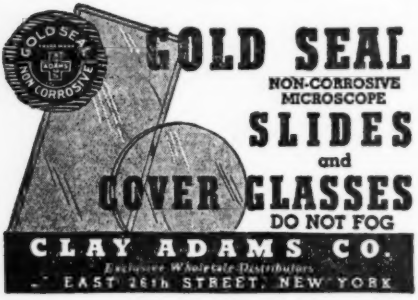
ABSTRACTS

USE IN WASSERMANN REACTION OF A UNIFORM, STABLE, DEHYDRATED COMPLEMENT: EAGLE, STRAUSS, AND STEINER; Amer. Jour. Clin. Path., Vol. 5:3, May, 1935.

This article should prove of great help to those working in serology. The authors have prepared a complement, having the properties of a lyophile, which is a serum repidly frozen and dehydrated in vacuo, giving a freely soluble product. First, the complement is frozen and dehydrated in a vacuum apparatus, recently made by Flosdorf and Mudd. The containers are then sealed in vacuo. This complement retains its full hemolytic activity for at least eight months in the ice-box, the longest period yet tested. It is restored by admitting distilled water through a rubber stopper with syringe and needle, the vacuum drawing in the water. It goes into solution quickly. This same process of dehydration can be used in almost indefinitely preserving amboceptor, Wassermann positive or negative sera. This dehydrated complement, restored to its original volume, aged over night in the ice-box and diluted, agreed perfectly with salted complement in 1,316 serum Wassermann tests.

DARK-FIELD ILLUMINATION IN THE DIAGNOSIS OF TUBERCULOSIS AND MALARIA: Charles Goosmann, M.D., Jour. Lab. and Clin. Med., Vol. 21:4, January, 1936.

This illustrated article demonstrates clearly the advantages of using the dark-field in the search for tubercle bacilli in sputum, and also the Plasmodium Malariae in Giemsa's stained blood films. Less time is consumed in positive findings, and there is less likelihood of missing a few scattered organisms.



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Koch discovered bacilli of cholera in 1883.

Nicolaier discovered tetanus bacillus in 1884.

Emmerich isolated colon bacillus in 1884.

American Society of Clinical Laboratory Technicians

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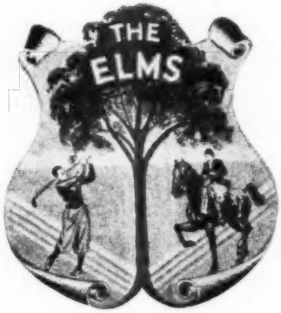

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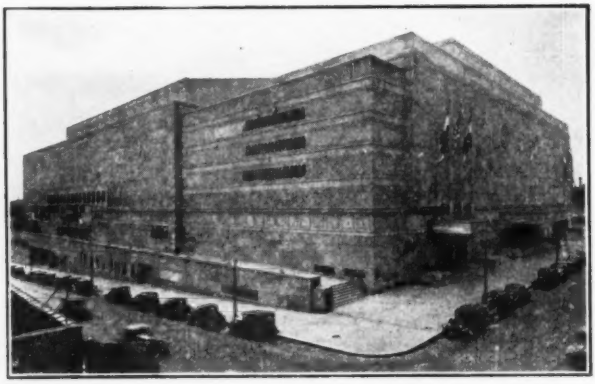

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EXECUTIVE SESSION

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MINUTES OF THE 1935 CONVENTION

APPOINTMENT OF COMMITTEES: Nominations, Resolutions

ANNOUNCEMENTS

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Intermission

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ADOPTION OF REPORTS

NEW BUSINESS: Voting on Proposed Changes in Constitution and By-Laws

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Presiding, FRIEDA CLAUSSEN

1. VERN L. FLANNERY, Baltimore, Md.:
   Some Sympathetic Factors in Thermo-Therapy
2. DR. GORDON R. KAMMAN, St. Paul, Minn.:
   Clinical Significance of Spinal Fluid Findings
3. DR. FRANK J. HECK, Mayo Clinic, Rochester, Minn.:
   The Morphological Diagnosis of Anemias

Intermission

4. HERMINE TATE, East Louisiana State Hospital, Jackson, La.:
   A Comparison of Kolmer Wassermann, Kahn and Johns Tests in Blood Serology
5. ROSE MATTHAEI, Houston, Texas (Texas Malaria Control):
   Technique of Examining Blood for Parasites

TUESDAY MORNING, MAY 12—9 TO 12 A. M.

Presiding, SISTER M. JOAN, R.S.M.

1. PHYLLIS STANLEY, Presbyterian Hospital, Newark, N. J.:
   The Preparation of Transudates for Histological Study
2. DR. F. M. JOHNS, Pres. A. S. C. P., New Orleans, La.:
   Cultivation of Fungi
3. DR. H. J. CORPER, National Jewish Hospital, Denver, Colo:
   The Cultivation of Tubercle Bacilli

Intermission

4. ANNETTE CALLAN, Temple University, Philadelphia, Pa.:
   Plain Facts About Blood Cultures
5. ANN SNOW, Medical School, University of Arkansas, Little Rock, Ark.:
   The Laboratory Recognition of Moniliae

TUESDAY AFTERNOON, MAY 12—2 TO 5 P. M.

Presiding, PHYLLIS STANLEY

1. BERNICE ELLIOTT, Omaha, Nebraska:
   The Folin-Berglund Method for the Quantitative Determination of Glucose in Urine
2. DR. PAUL ROTH, Battle Creek Sanatarium, Battle Creek, Mich.:
   Basal Metabolism
3. DR. M. BODANSKY, University of Texas, Galveston, Texas:
   The Scope of Chemical Pathology

Intermission

4. DR. I. DAVIDSOHN, Mount Sinai Hospital, Chicago, Ill.:
   Management of the Clinical Laboratory
5. ALMA E. TILLOTSON, Chicago, Ill.:
   Rapid Method for Preparing Paraffin Sections

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Presiding, CHRISTINE SEGUIN

1. DR. RALPH E. DUNCAN, Kansas City, Mo.:
   The Technician and Laboratory Patrons
2. DR. KANO IKEDA, St. Paul, Minn.:
   Registration of Technicians
3. VIVIAN HERRICK, Cicero, Ill.:
   The Heritage of the Clinical Laboratory

Intermission

REPORT OF NOMINATION COMMITTEE
REPORT OF RESOLUTIONS COMMITTEE
UNFINISHED BUSINESS

WEDNESDAY AFTERNOON, MAY 13—2 TO 5 P. M.

ELECTION OF OFFICERS
VISITS TO AMERICAN MEDICAL ASSOCIATION EXHIBITS IN KANSAS CITY

WEDNESDAY EVENING, MAY 13—7:30 P. M.

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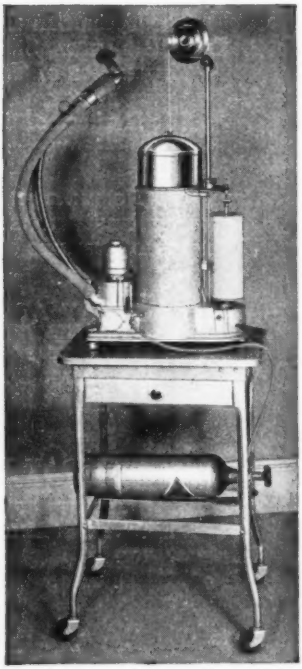

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